THE EFFECT OF 2,4-DICHLOROBENZYLTRIBUTYLPHOSPHONIUM CHLORIDE AND 2-CHLOROETHYLTRIMETHYLAMMONIUM CHLORIDE ON GROWTH, FLOWERING AND CHEMICAL COMPOSITION OF *CHRYSANTHEMUM MORIFOLIUM* 'BLUECHIP'

By

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INTRODUCTION

The following statement was made by Dr. C. L. Lefebvre (35), Director of Plant Science Division of SCRS: "There is current widespread interest in the possible use of growth regulators as growth retardants for floricultural and ornamental plants. This is a relatively new field of interest, and fundamental research on the mode of action of such chemicals will help to establish a firm scientific base for research in this area."

Compounds that produce desirable effects on ornamental plants, e.g., dwarfing, early flowering, drought and cold resistance would greatly benefit the plant industry. Growth regulators affect the size, form, rate of growth, flowering and environmental response of plants. The possibility of improving plant grade and size, reducing time to flowering and increasing flower number through uses of these compounds needs exploring. A study of the effects of growth regulators on the chemical constituents of plants is necessary to provide basic knowledge for a better understanding of their mode and site of action.

Many commercially produced compounds show promise as practical growth retardants. The two chemicals chosen for this study were 2,4-dichlorobenzyltributylphosphonium chloride (Phosfon) and 2-chloroethyltrimethylammonium chloride (CCC). Phosfon has been reported to be more active on a molar basis than CCC, to have a longer residual effect in the soil and to produce more permanent effects on the plants (2).

This work was designed to study effects of Phosfon and CCC on certain phases of growth, flowering and chemical composition of Chrysanthemum morifolium 'Bluechip.' Chrysanthemums were chosen because they can be obtained in a vegetative or flowering state at any time by proper photoperiod treatment and also because they have been used previously as index plants for growth retardant studies (2,3,6,8,52).

LITERATURE REVIEW

Structure of growth retardant compounds

CCC is a quaternary ammonium compound, $Cl(CH_2)_2N(CH_3)_3Cl$, and Phosfon a quaternary phosphonium compound, $C_6H_6Cl_2CH_2P(C_4H_9)_3Cl$. Three of the four radicals in each compound are similar, methyl in CCC, butyl in Phosfon. 2-Chloroethyl (CCC) and 2,4-dichlorobenzyl (Phosfon) form the fourth radical. Quaternary ammonium compounds contain a nitrogen atom with four functional groups, while carbamates, which also affect plant growth, are formed from carbamic acid, NH_2COOH.

Halevy and Cathey (24) tested several carbamate compounds on the growth of cucumber seedlings. Ring carbamates with five or six carbons retarded growth of radicles and hypocotyls at concentrations of 1.9 to 7.5×10^{-3} M. Carbamates with phenyl or butyl groups were toxic. Methyl substituted carbamates or six membered ring carbamates containing oxygen retarded growth of hypocotyls at high concentrations, but had no effect on the growth of the radicle. Minor molecular changes profoundly altered the effectiveness of the compounds. Krewson et al. (31) studied quaternary ammonium and related compounds and found that 11 compounds which reduced growth contained two nitrogen atoms per molecule, one quaternary and one carbamate. Activity was enhanced by substitution of methyl, isopropyl or tertiary butyl on the aromatic ring suggesting an increase in activity due to increased molecular size.

Tolbert (59) found the trimethyl ammonium cation essential for stem reduction of wheat by substituted cholines. For optimum activity the

carbon chain to which the halide is covalently bonded should contain two carbons. CCC is structurally related to choline in that the hydroxyl group of choline had been replaced by a halogen.

The tributyl quaternary phosphonium cation of the phosphoniums is reported to be necessary for activity and any substitution of shorter or longer alkyl groups or phenyl groups for even one butyl group produced nearly inactive compounds. Optimum activity was produced by substitution on the fourth position of the benzene ring (4).

There is no evidence that these compounds occur naturally.

Vegetative and flowering effects

The most noticeable effect of growth retardants is a reduction in height of treated plants. Tolbert (60) observed shorter and thicker stems of wheat when treated with CCC and also treated plants were more uniform in height. Wittwer and Tolbert (69) suppressed vegetative expansion of tomato and of both genetically dwarf and normal lettuce plants with application of CCC. Camellia, zinnia, holly, sunflower, chrysanthemum and azalea are some ornamental plants that have responded to treatment with growth retardants (2,5,10,19,21,36,38).

Flowering may be delayed by growth retardants (70), but some authors report increases in flower number and decreases in time to flowering (38,50). Phosfon increased the time to flowering and decreased flower diameter of chrysanthemum (2).

Phosfon and CCC applied to azaleas in a non-flowering environment caused initiation of flower buds promptly after their application as soil drenches (57,58). Tomato plants treated with CCC flowered 3-10 days earlier than non-treated ones and more prolific fruiting of marketable tomatoes was promoted (69). CCC and Phosfon also stimulated flower bud development of camellia (19).

Growth retardants were reported to shorten and thicken internodes (7,10,14,59), reduce fresh and dry weight of stems but not to reduce leaf weight or area (2).

Phosfon and CCC often caused leaves to become darker green (4,8,9), although some levels of growth retardants produced chlorosis (19). Cathey (2) treated chrysanthemums with growth retardants and various levels of nitrogen, potassium and phosphorus and found that increase in green color was directly related to growth regulators. Leaves of plants receiving growth retardants were darker than those on untreated plants at the same level of fertilization. Darker green leaves resulting from Phosfon treatments have been observed on petunia, salvia and phlox (6).

Humphries (28) found that chlorophyll content per unit area and per leaf was increased in tobacco plants treated with CCC. Nitrogen was increased in the leaf and decreased in the stem.

Salt tolerance on soybean was increased by treatment with growth retardants (39). When 5 grams of a 5-10-5(N-P_2O_5-K_2O) fertilizer were applied per three-inch pot, plants treated with Phosfon and CCC remained healthy but untreated plants were killed.

Other effects attributed to growth retardants are reduction of micro-organisms that cause foliar discoloration (40), reduction of external infestations of mites (39), a slight reduction of root systems (9) and complete inhibition of root growth of cuttings (8).

Cathey and Stuart (10) tested 55 species of plants and reported that response to treatment with CCC was generally noticeable at the end of one week, while response to Phosfon took two to three weeks.

Influence of environment

Intensity and duration of light, temperature and maturity of the plants affect their responses to growth retardants. Dwarfing of tomato with CCC decreased with longer nights, higher temperatures and more intense sunlight (69).

Petunia plants grown on a 16 hour photoperiod with a night temperature of 80°F. were retarded less by Phosfon than were plants grown at night temperatures of 70°F. or less. As temperature increased from 50° to 80°F. there was an increase in stem length at the same concentration of Phosfon and young plants were more responsive than old plants (9). Another growth retardant, maleic hydrazide (MH), was shown by Greulach and Atchison (20) to be more effective on young than on older plants of Allium cepa.

Another quaternary ammonium compound, 4-hydroxyl-5-isopropyl-2-methyl phenyl trimethylammonium chloride, 1-piperdine carboxylate (Amo-1618), was more effective at 80° than 40°F. when chrysanthemum cuttings were soaked for 24 hours (8). However, temperature had no effect when cuttings were soaked for 5 seconds. Cathey and Stuart (10) reported that Phosfon was more effective in the summer whereas CCC was less effective. Tolbert (61) also reported CCC to be more effective at low temperatures. Maximum inhibition of growth and flowering of chrysanthemums by Amo-1618 occurred when plants were treated at the beginning of short photoperiods (3).

Chemical stability

Growth retardants appear very stable. Amo-1618 was reported to remain effective in the soil for as long as ten years and Phosfon for more than a year. CCC persists for four weeks (10). Riddell et al. (51) noted that N-dimethylamino maleamic acid (CO 11) added to soil before

tuber production of potatoes, caused resulting tubers to produce short plants when they were planted the following spring. Effects of growth retardants were transmitted through three generations of Alaska peas (41).

Cell division and enlargement

Greulach and Atchison (20) treated roots of Allium cepa with MH and found that low concentrations inhibited cell division, moderate concentrations inhibited cell enlargement and division, and high concentrations of 1,000 and 2,000 parts per million killed the plant. MH had been shown to suppress metaxylem and secondary xylem formation of sunflower (21).

Halevy and Cathey (24) concluded that quaternary ammonium compounds retarded cell enlargement and cell division of cucumber seedlings. Haber and White (22) postulated that MH affected mitosis in a system where gibberellic acid (GA) did not and MH did not affect cell expansion in a system where GA was active.

Wheaton (65) treated Alaska pea seeds with Amo-1618 and noticed that after 14 days pith cells were shorter in treated plants than untreated plants. Zeevaart (70) found that CCC treated plants had one-third as many cells at maturity as untreated plants.

Similarities of growth retardants and environmental factors

Cabler (1) showed that Phosfon and CCC could partially substitute for high light intensities. Grass grown under low light intensities and treated with these chemicals was not etiolated which presented a marked contrast to the etiolated controls. Downs and Cathey (14) also reported that Amo-1618 produced plants under low light intensities that were similar to those grown under high light intensities and retardation induced by

Amo-1618 was equally as great on redirradiated as dark grown plants. Tolbert (60) found that CCC caused wheat plants to act opposite to GA since GA produced plants which appeared as though they had received too little light, while CCC treated plants were darker green in appearance with shorter and broader leaves.

Mode of action

Many researchers have attempted to determine the mode of action of growth retardants. One approach used has been to study interactions between growth retardants and growth promoters such as GA or indoleacetic acid (IAA).

In 1960 Tolbert (60) reported that CCC and GA were mutually antagonistic and suggested that the action of CCC altered the developmental pattern rather than growth rate. Cathey (5) demonstrated mutual antagonism of GA and Amo-1618 on growth and flowering of chrysanthemums. Conrad and Saltman (12) tested the interaction of GA and allyl trimethylammonium bromide (AMAB) on growth of Ulothrix. They found that AMAB promoted growth at low concentrations and inhibited growth at high concentrations and that there was a mutual competitive inhibition on growth between GA and AMAB. They felt that the inhibitors either combined directly with the growth substance binding site or functioned in a partially competitive system in which the inhibitor affected the affinity of an enzyme for substrate. Kawahara et al. (30) also reported that growth retardants and GA were mutually antagonistic. Lockhart (37) called Phosfon and CCC antigibberellins and found that these compounds exerted inhibiting effects in the stem rather than in the roots and concluded that Phosfon and CCC retarded stem elongation by partially blocking the system which provided active GA to the growth mechanism.

Tolbert (60) showed that application of CCC to wheat caused early tillering, but if GA was applied simultaneously with CCC early tillering was prevented. Early tillering was not reversed when GA was applied after tiller buds had been initiated and started growth. Wittwer and Tolbert's (68) studies with a variety of biological systems confirmed the observation that CCC and GA induce opposite growth responses. He concluded that their chemical structures were so different they were not involved in the same growth systems.

Kruaishi and Muir (33) studying the influence of CCC, Phosfon, GA and IAA on various growth systems, reported that inhibition of CCC and Phosfon was greatest in combination with high concentrations of GA or IAA on leaf growth of bean. Inhibitory effects of CCC on coleoptile growth was overcome by higher concentrations of IAA but not by GA. Stem segments of Alaska peas whose growth was retarded by CCC did not respond to GA but increased in length when IAA was added. Diffusible auxin from stem apices of pea plants retarded by CCC was only one-seventh as much as diffusible auxin from normal plants. Evidence indicated that growth retarding effects of CCC was due to low auxin levels in treated plants. Kuraishi and Muir (32) earlier reported that treatment of plants with GA resulted in increased levels of endogenous auxin in several plants.

Several workers have studied peroxidase and IAA oxidase activities in plants treated with growth retardants. Halevy (23) found GA caused hypocotyl tips and cotyledons of cucumber seedlings to exhibit less and Amo-1618 to exhibit more peroxidase and IAA oxidase activity. Stimulation of peroxidase and IAA oxidase activity of tissue was found with five growth retardants. He proposed that growth retarding chemicals affected

plant growth by interacting with GA on either IAA oxidase or its co-factors and inhibitors and thus altered auxin level of tissues. MH did not affect IAA oxidase and did not alter auxin content of plant tissue (48). Red light was credited with controlling plant growth by influencing IAA oxidase (44). Downs and Cathey (14) suggested that Amo-1618 action was interrelated with the action of GA but not with red-infrared photoreaction. They reported that light increased cell development while GA stimulated cell division and enlargement. Amo-1618 apparently acted as a mitotic inhibitor in subapical meristems and probably inhibited through an interference with naturally occurring GA.

CCC is known to be an inhibitor of choline esterase in vitro, but there is no evidence of choline esterase occurring in plants. Choline is involved in lipid metabolism and methylation reactions. Thus, CCC might have a function in lipid metabolism. The physiology of growth retarding chemicals has recently been reviewed by Cathey (7).

Amino acids

Amino acids are amino and carboxyl derivatives of alkanes, whose general formula is $R\text{-}CH(NH_2)\text{-}COOH$. Amino acids apparently are building units of proteins and more than 20 alpha types have been isolated from hydrolytic products of protein material.

Chemical pathways of plant nitrogen metabolism may be divided roughly into four areas: (a) assimilation of nitrogen, (b) formation and interconversion of amino acids, (c) synthesis of amides, peptides and other simple nitrogenous substances and (d) formation and degradation of proteins and nucleic acids (64). However, there are unanswered questions on the position of amino acids in nitrogen metabolism. Some researchers have suggested that amino acids are formed chiefly as a result of nitrogen

assimilation and are direct precursors of protein. Others have suggested that amino acids are only products of protein breakdown, the protein being formed from carbohydrate skeletons and nitrogen from such donors as ammonia, glutamine and glutamic acid. Hellebust and Bidwell (25) felt that amino acids were products of respiration breakdown since during photosynthesis a large proportion of carbon used in protein synthesis came directly from newly photoassimilated carbon and bypassed the bulk of soluble amino acids.

Many factors affect the level of amino acids in plants. Steward et al. (56) found the ratio of alcohol soluble to alcohol insoluble nitrogen was greater in resting than proliferating tissue. These differences were due to a low content of amino acids and amides in the growing tissue, especially asparagine, glutamine and arginine. The most common amino acids in the soluble extract were glutamine, asparagine, arginine, gamma amino butyric acid, glutamic acid and alanine. Asparagine, glutamine and arginine are all nitrogen storage compounds.

Plaisted (49) reported that the amount of protein per leaf increased during late spring and early summer, remained constant during the summer and decreased as the leaf reached old age. Soluble nitrogen had a similar trend.

Pauli and Mitchell (47) found a considerable increase in amino acid content when wheat grown at 70°F. was moved to a 35°F. temperature. There was a high correlation between amino acid increase and winter hardiness.

Mizusaki et al. (43) observed that tobacco synthesized proline rapidly during the day but slowly during the night. Proline constituted a large percentage of the total amount of free amino acids of young

leaves, but gamma amino butyric acid was high in older leaves. DeKock et al. (13) found that significantly larger amounts of free amino acids were associated with tissue in which chlorosis was present. Several workers have reported varying amounts of amino acid content due to inorganic element deficiencies (11,16,17,18,29,42). Differences in soluble nitrogen content of tobacco was caused primarily by aspartic acid, glutamic acid, proline, serine and the respiratory amides. Proline was highest in magnesium deficient cultures (34).

MATERIALS AND METHODS

This experiment was initiated March 19, 1964, to test effects of two levels each of Phosfon and CCC on growth, flowering and chemical composition of Chrysanthemum morifolium 'Bluechip,' a short-day, nine-week variety. Treatments were replicated three times with three plants per pot an experimental unit. Rooted cuttings were potted February 14, in 5-inch clay pots in a mixture of two-thirds sandy soil and one-third peat. At the time the plants were pinched, 60 watt incandescent lights were placed 6 feet above the plants 4 feet apart. Lights were turned on daily from 11:00 P.M. to 2:00 A.M. The long day treatment was initiated on February 23, and extended to March 25. After this date short-day treatments were initiated by placing black sateen cloth over the plants from 5:00 P.M. to 8:00 A.M.

Chemical treatments were applied to the soil on March 19. The actual amounts of Phosfon and CCC applied to a pot were 0.3 and 0.6 ml. of a 10 per cent solution and 2.5 and 5.0 ml. of an 11.8 per cent solution, respectively. In all cases these concentrated solutions were diluted to 50 ml. with water before they were applied. Thus application per pot was either 7.5×10^{-5} or 1.5×10^{-4} moles of Phosfon or 1.9 or 3.8×10^{-3} moles of CCC. The plants had been watered thoroughly the day before application of Phosfon and CCC.

Growth measurements and samples for analyses were taken five different times from March 19, to March 25. Samples were taken immediately before chemical treatments (S-1), and after treatment 6 hours (S-2),

24 hours (S-3), three days (S-4) and six days (S-5). Growth and flower data were taken May 25, at the termination of the experiment. Growth measurements included total height, stem diameter and length of fourth internode from pinch and fresh and dry weight of stems and leaves. Flower data included number of flowers fully opened, number of buds showing color and flower diameter. The latter was determined by measuring the five largest flowers in each experimental unit.

For inorganic analysis mature leaf and stem samples were dried at 65°C. for 48 hours, then ground in a Wiley Mill and stored in air tight containers until used for analysis. One gram samples of the dried tissue were ashed in a muffle furnace at 500°C. The residue was dissolved in 15 ml. of 50 per cent HCl, evaporated to dryness for silicate removal and brought to 100 ml. with 0.1 N HCl. Aliquots of this extract were then analyzed for phosphorus, potassium, calcium and magnesium. A Beckman Model DU Flame Spectrophotometer was used for magnesium, potassium and calcium determinations after the solutions of ashed material were passed through a column of Dowex 1-X8 anion exchange resin for removal of interfering anions (26). Phosphorus was determined by the ammonium molybdate-amino-napthal sulfonic acid procedure utilizing the Bausch and Lomb Spectronic 20 Colorimeter (27). Nitrogen was determined by the micro Kjeldahl method (66).

Ten grams of recently matured leaves were taken for amino acid analysis and placed immediately in 100 ml. of hot 80 per cent alcohol, then stored in a -3°C. freezer until analyzed for free amino acids. For analysis the leaves were ground in a Waring Blender for three minutes, filtered and washed with alcohol. The filtrate was reduced under vacuum at 50°C. to approximately 10 ml., washed with chloroform and passed

through Dowex 50-X8, 100-200 mesh, H^+ form. The Dowex resin was washed with 20 ml. of water, 15 ml. of N NH_4OH, 15 ml. of 3 N NH_4OH and 15 ml. of water to elute amino acids.

The eluate was collected after the addition of NH_4OH to the resin column, dried under reduced pressure at 45°C. after which 0.3 ml. of N HCl and 1.7 ml. of 10 per cent isopropanol were added. Twenty lambda of this solution was spotted on a Whatman No. 1 chromatographic filter paper and chromatographed two dimensionally using butanol/acetic acid/water, 5/1/5, then phenol/water, 4/1. The papers were equilibrated for at least four hours before placing solvent in the trough. Ten ml. of 3 N NH_4OH were placed in the bottom of the phenol/water chamber. The papers were dried in a hood, dipped in 0.4 per cent ninhydrinacetone solution and dried for 30 minutes at 60°C. Individual amino acids and amide spots were cut out along with a blank spot, cut into small pieces and placed in test tubes containing 5 ml. of 50 per cent ethyl alcohol for 30 minutes. The densities of the purple solutions were read at 570 millimicrons on the Bausch and Lomb Spectronic 20 Colorimeter. Proline and asparagine solutions were read at 360 millimicrons on the Beckman DU Spectrophotometer.

Standards for each amino acid were determined by placing known quantities of individual amino acids and amides in an aqueous solution, reducing the volume, passing the reduced solution through the resin, drying and chromatographing the eluate as outlined above.

To test reliability of the above procedure chrysanthemum leaves were split at the midrib, the duplicate samples analyzed and the quantitative results compared. Duplications were replicated four times.

Comparisons indicated that the procedure was quantitatively reliable within 5 per cent.

All data were statistically checked for reliability by the analysis of variance as outlined by Snedecor (53).

RESULTS

Treatment had no effect on growth during the first six days, although there were increases in each measurement during this time (Table 1).

At the termination of the experiment treatment with growth retardants influenced vegetative and flower measurements. CCC had no effect on plant height, but Phosfon greatly reduced height with the high level reducing growth more than the low level (Table 2). Diameter of the fourth internode was unaffected by treatment with growth retardants. The fourth internode of Phosfon treated plants was longer than the fourth internode of the control or CCC treated plants. Plants treated with the low level of Phosfon had a longer internode than plants treated with the high level. Phosfon treated plants had fewer flower buds and fully opened flowers than the control or CCC treated plants. The flower diameter of plants treated with Phosfon was smaller than the flower diameter of the control or CCC treated plants. The high level of Phosfon caused plants to produce smaller flowers than plants treated with the low level. Treatment with CCC did not affect flower diameter.

Chlorosis Index

The high CCC level caused interveinal chlorosis on leaves within four days after treatment (Table 3). At the termination of the experiment there was no difference in leaf color between non-treated and treated plants.

Chemical Analyses

Inorganic

Treatment had no effect on nitrogen, potassium, phosphorus, calcium or magnesium content of stems (Table 4).

One day after treatment leaves of plants receiving growth retardants contained less per cent dry weight nitrogen than control plants (Table 5). Six days after treatment there was more nitrogen in plants treated with CCC than in the other plants.

After treatment with Phosfon the potassium content of mature leaves increased at the sixth hour (Table 5). However, one day after treatment the control and CCC treated plants contained more potassium than did the Phosfon treated plants and by the sixth day, treatment had no effect on potassium content.

Treatment with the high CCC level caused an increase in magnesium and calcium content of leaves six days after treatment. Plants treated with the low Phosfon level contained less phosphorus six days after treatment than those treated with the high level of CCC (Table 6). There was an increase of iron in plants six days after treatment with the high level of Phosfon and both levels of CCC (Table 6).

Amino acids

Phosfon caused an increase in the content of alanine in the leaves six hours after treatment (Table 7).

CCC treated plants contained more asparagine than the control or Phosfon treated plants on the first and sixth day after treatment (Table 7).

There was less aspartic acid in the control plants one day after treatment than in plants treated with growth retardants (Table 7). The

sixth day after treatment, CCC treated plants contained more aspartic acid than Phosfon treated plants, which contained more aspartic acid than the control plants.

Control plants contained less glutamine than Phosfon treated plants one day after treatment (Table 7). Six days after treatment CCC treated plants contained more glutamine than control or Phosfon treated plants.

There was no difference between glutamic acid levels one day after treatment, but on the sixth day, CCC treated plants contained more glutamic acid than both the control and Phosfon treated plants (Table 7).

On the first and sixth day after treatment, CCC treated plants had more glutamic plus aspartic acid than Phosfon treated plants which contained more of these acids than control plants (Table 8). Phosfon treated plants contained more of the amides, asparagine and glutamine than the control or CCC treated plants one day after treatment. CCC caused plants to contain more of the amides than the control or Phosfon treated plants six days after treatment.

There was a large increase of proline in plants six hours after treatment at the high Phosfon level and low CCC level. Plants treated with the high level of Phosfon contained more than plants treated with the low CCC level (Table 9). Plants treated with the high CCC level did not show an increase of proline until 24 hours after treatment. There was a large increase in serine six hours after treatment at the high CCC level.

Treatments with growth retardants caused an increase in total free amino acids six hours after treatment with the high levels causing a larger increase than low levels (Table 10). One day after treatment

plants treated with the high level of Phosfon and both levels of CCC contained more free amino acids than the non-treated and plants treated with the low level of Phosfon. Six days after treatment there were more free amino acids in plants treated with CCC than non-treated plants.

The predominant free amino acids found in leaves of chrysanthemums used in this experiment were glutamine, glutamic acid, aspartic acid, serine, proline and alanine (Tables 12, 14, 16, 18). The total free amino acids in the leaves expressed as micromoles per gram of fresh weight can be found in Tables 11, 13, 15, 17.

Table 1. Vegetative measurements of Chrysanthemum morifolium 'Bluechip' taken immediately before treatment with Phosfon and CCC and 6 days after treatment

	Before treatment	Six days after treatment			
		10^{-5} moles of chemical per 5-inch pot			
		0	P-1(7.5)	P-2(15)	C-1(190)	C-2(380)
Height (cm.)	23.6a	29.8b	28.5b	29.0b	28.3b	29.7b
Stem, fresh weight (gm.)	4.7a	8.5b	7.3b	8.3b	8.8b	8.7b
Stem, dry weight (gm.)	1.0a	1.8b	1.7b	2.0b	1.7b	1.8b
Leaves, fresh weight (gm.)	12.5a	17.3b	16.7b	16.5b	20.3b	21.2b
Leaves, dry weight (gm.)	2.0a	3.3b	3.5b	3.2b	3.7b	3.7b
Diameter of 4th internode (mm.)	2.4a	3.1b	2.9b	3.0b	3.2b	3.1b
Length of 4th internode (cm.)	2.6a	3.9b	3.6b	4.0b	3.7b	4.0b

Means within horizontal rows followed by same letter are not significantly different at the 5 per cent level.

Table 2. Vegetative and flower measurements of Chrysanthemum morifolium 'Bluechip' taken 67 days after treatment with Phosfon and CCC

	10^{-5} moles of chemical per 5-inch pot				
	0	P-1(7.5)	P-2(15)	C-1(190)	C-2(380)
Height (cm.)	72.1a	61.6b	54.9c	71.5a	68.2a
Diameter of 4th internode (mm.)	3.5a	3.5a	3.4a	3.5a	3.5a
Length of 4th internode (cm.)	3.7c	4.4a	4.0b	3.7c	3.8c
Number of flower buds	34.0b	29.8a	28.2a	36.4b	33.0b
Number flowers fully opened	20.4b	8.2a	5.1a	17.2b	16.5b
Flower diameter (cm.)	6.4a	4.5b	3.2c	6.0a	5.7a

Means within horizontal rows followed by same letter are not significantly different at the 5 per cent level.

Table 3. Leaf chlorosis ratings of Chrysanthemum morifolium 'Bluechip' treated with Phosfon and CCC

Days after treatment	10^{-5} moles of chemical per 5-inch pot				
	0	P-1(7.5)	P-2(15)	C-1(190)	C-2(380)
4	3.0a	3.0a	3.0a	3.0a	1.1b
66	2.5a	2.4a	2.8a	2.6a	2.5a

Means within horizontal rows followed by same letter are not significantly different at the 5 per cent level. Rating scale: 1-chlorotic, 2-light green, 3-dark green.

Table 4. Inorganic analysis of stems of Chrysanthemum morifolium 'Bluechip' per cent dry weight on sixth day after treatment with Phosfon and CCC

	10^{-5} moles of chemical per 5-inch pot				
	0	P-1(7.5)	P-2(15)	C-1(190)	C-2(380)
Nitrogen	1.5a	1.4a	1.5a	1.5a	1.7a
Phosphorus	0.4a	0.4a	0.4a	0.4a	0.5a
Potassium	2.8a	2.6a	2.3a	2.5a	3.2a
Magnesium	0.6a	0.6a	0.6a	0.6a	0.6a
Calcium	0.2a	0.2a	0.2a	0.2a	0.2a

Means within horizontal rows followed by same letter are not significantly different at the 5 per cent level.

Table 5. Effect of Phosfon and CCC on per cent nitrogen and potassium on a dry weight basis in leaves of Chrysanthemum morifolium 'Bluechip'

Days after treatment	Control	Phosfon	CCC
		Nitrogen	
1	4.2a	3.8b	3.7b
6	3.4a	3.2a	4.0b
		Potassium	
1/4	4.1b	4.7a	3.9b
1	4.7b	3.7a	4.4b
6	3.1a	2.8a	3.1a

Means within horizontal rows followed by same letter are not significantly different at the 5 per cent level.

Table 6. Effect of Phosfon and CCC on per cent calcium, magnesium, iron and phosphorus on a dry weight basis in leaves of Chrysanthemum morifolium 'Bluechip' 6 days after treatment

	10^{-5} moles of chemical per 5-inch pot				
	0	P-1(7.5)	P-2(15)	C-1(190)	C-2(380)
Magnesium	0.35a	0.30a	0.33a	0.32a	0.45b
Calcium	0.36a	0.35a	0.38a	0.37a	0.53b
Phosphorus	0.36ab	0.31a	0.38ab	0.37ab	0.45b
Iron	0.021a	0.020a	0.024b	0.024b	0.024b

Means within horizontal rows followed by same letter are not significantly different at the 5 per cent level.

Table 7. Effects of Phosfon and CCC on alanine, asparagine, aspartic acid, glutamine and glutamic acid in micromoles per gram fresh weight in leaves of Chrysanthemum morifolium 'Bluechip'

Days after treatment	Control	Phosfon	CCC
		Alanine	
1/4	1.05a	1.81b	1.16a
		Asparagine	
1	1.09a	0.96a	1.24b
6	0.73a	0.82a	1.19b
		Aspartic acid	
1	1.43a	2.59b	2.67b
6	0.63a	0.35b	0.92c
		Glutamine	
1	4.87a	6.30b	5.21ab
6	0.35a	0.28a	1.13b
		Glutamic acid	
1	2.88a	2.75a	3.14a
6	1.75a	1.96a	2.84b

Means within horizontal rows followed by same latter are not significantly different at the 5 per cent level.

Table 8. Effect of Phosfon and CCC on totals of asparagine plus glutamine and aspartic plus glutamic acid in micromoles per gram fresh weight in leaves of Chrysanthemum morifolium 'Bluechip'

Days after treatment	Control	Phosfon	CCC
		Asparagine plus glutamine	
1	5.97a	7.26b	6.46a
6	1.08a	1.10a	2.32b
		Aspartic plus glutamic acid	
1	4.31a	5.34b	5.81c
6	2.38a	3.31b	4.76c

Table 9. Effect of Phosfon and CCC on proline and serine in micromoles per gram fresh weight in leaves of Chrysanthemum morifolium 'Bluechip'

Days after treatment	10^{-5} moles of chemical per 5-inch pot				
	0	P-1(7.5)	P-2(15)	C-1(190)	C-2(380)
			Proline		
1/4	1.16a	1.37a	3.37c	2.29b	1.21a
1	0.92a	0.66a	0.66a	0.96a	0.70b
			Serine		
1/4	1.73a	2.05a	2.50a	2.88a	5.44b

Means within horizontal rows followed by same letter are not significantly different at the 5 per cent level.

Table 10. Effect of Phosfon and CCC on total free amino acid content in micromoles per gram fresh weight, of leaves of Chrysanthemum morifolium 'Bluechip'

Days after treatment	10^{-5} moles of chemical per 5-inch pot				
	0	P-1(7.5)	P-a(15)	C-1(190)	C-2(380)
1/4	16.21a	20.95b	26.75d	22.77bc	25.09dc
1	13.99a	14.67a	17.77b	17.88b	16.80b
6	6.91a	8.91ab	7.54ab	10.35b	13.04b

Means within horizontal rows followed by same letter are not significantly different at 5 per cent level.

Table 11. Effect of Phosfon and CCC on free amino acids in micromoles per gram fresh weight in leaves of Chrysanthemum morifolium 'Bluechip'

	Before treatment	Six hours after treatment				
		10^{-5} moles of chemical per 5-inch pot				
		0	P-1(7.5)	P-2(15)	C-1(190)	C-2(380)
Alanine	1.05	1.05	2.10	1.52	1.10	1.21
Arginine	0.44	0.15	0.15	0.30	0.15	0.15
Aspartic acid	2.58	1.66	2.52	3.21	2.29	2.98
Asparagine	1.64	1.00	0.64	0.73	1.10	1.28
Glutamic acid	3.78	2.38	1.99	3.59	2.98	3.50
Glutamine	10.16	5.80	8.46	9.59	7.80	7.80
Gamma amino butyric acid	0.17	0.37	0.43	0.57	0.37	0.33
Isoleucine	1.14	0.62	0.81	0.95	1.09	0.76
Proline	0.96	1.16	1.37	3.37	2.29	1.21
Serine	1.79	1.73	2.05	2.50	2.88	5.44
Threonine	0.30	0.15	0.15	0.15	0.30	0.15
Valine	0.28	0.14	0.28	0.28	0.42	0.28

Table 12. Effect of Phosfon and CCC on individual free amino acids (expressed as per cent of the total free amino acids) in leaves of Chrysanthemum morifolium 'Bluechip'

	Before treatment	Six hours after treatment				
		10^{-5} moles of chemical per 5-inch pot				
		0	P-1(7.5)	P-2(15)	C-1(190)	C-2(380)
Alanine	4.3	6.5	10.0	5.7	4.9	4.8
Arginine	1.8	0.9	0.7	1.1	0.7	0.6
Aspartic acid	10.6	10.2	12.0	12.0	10.1	11.9
Asparagine	6.8	6.2	3.0	2.7	4.8	5.1
Glutamic acid	15.6	14.7	9.5	13.4	13.1	13.9
Glutamine	41.8	35.8	40.4	35.8	34.2	31.1
Gamma amino butyric acid	0.7	2.3	2.0	2.1	1.6	1.4
Isoleucine	4.7	3.8	3.9	3.6	4.8	3.0
Proline	4.0	7.2	6.5	12.6	10.1	4.8
Serine	7.4	10.7	9.8	9.3	12.6	21.7
Threonine	1.2	0.9	0.7	0.6	1.3	0.6
Valine	1.2	0.9	1.3	1.0	1.8	1.1

Table 13. Effect of Phosfon and CCC on free amino acids, micromoles per gram fresh weight, in leaves of Chrysanthemum morifolium 'Bluechip' one day after treatment

	10^{-5} moles of chemical per 5-inch pot				
	0	P-1(7.5)	P-2(15)	C-1(190)	C-2(380)
Alanine	0.84	0.74	1.00	1.05	0.89
Arginine	0.15	0.44	0.15	0.15	0.15
Aspartic acid	1.43	2.81	2.23	2.75	2.58
Asparagine	1.10	0.91	1.00	1.19	1.28
Glutamic acid	2.88	2.38	3.12	3.69	2.60
Glutamine	4.88	4.93	7.67	5.10	5.32
Gamma amino butyric acid	0.13	0.13	0.17	0.20	0.20
Isoleucine	0.28	0.24	0.33	0.62	0.36
Proline	0.92	0.46	0.66	0.96	1.70
Serine	1.09	1.34	1.15	1.73	1.41
Threonine	0.15	0.15	0.15	0.30	0.15
Valine	0.14	0.14	0.14	0.14	0.14

Table 14. Effect of Phosfon and CCC on individual free amino acids expressed as per cent of the total free amino acids in leaves of Chrysanthemum morifolium 'Bluechip' one day after treatment

	10^{-5} moles of chemical per 5-inch pot				
	0	P-1(7.5)	P-2(15)	C-1(190)	C-2(380)
Alanine	6.0	5.0	5.6	5.9	5.3
Arginine	1.1	3.0	0.8	0.8	0.9
Aspartic acid	10.2	19.2	12.5	15.4	15.4
Asparagine	7.9	6.2	5.6	6.6	7.6
Glutamic acid	20.6	16.2	17.6	20.6	15.5
Glutamine	34.9	33.6	43.2	28.5	31.7
Gamma amino butyric acid	0.9	0.9	1.0	1.1	1.2
Isoleucine	2.0	1.6	1.9	3.5	2.1
Proline	6.6	3.1	3.7	5.4	10.1
Serine	7.8	9.1	6.5	9.7	8.4
Threonine	1.1	1.0	0.8	1.7	0.9
Valine	1.0	1.0	0.8	0.8	0.8

Table 15. Effect of Phosfon and CCC on free amino acids, micromoles per gram fresh weight, in leaves of Chrysanthemum morifolium 'Bluechip' three days after treatment

	10^{-5} moles of chemical per 5-inch pot				
	0	P-1(7.5)	P-2(15)	C-1(190)	C-2(380)
Alanine	1.21	0.53	0.74	0.84	0.89
Arginine	0.15	0.15	0.15	0.15	0.15
Aspartic acid	1.60	2.06	1.55	3.04	2.00
Asparagine	0.82	0.82	0.91	0.73	1.00
Glutamic acid	2.36	2.27	2.88	3.17	2.60
Glutamine	0.74	0.35	1.00	0.65	0.87
Gamma amino butyric acid	0.27	0.20	0.17	0.33	0.17
Isoleucine	0.33	0.28	0.48	0.33	0.38
Proline	0.54	0.46	0.71	1.12	1.12
Serine	0.77	1.15	1.09	1.27	0.64
Threonine	0.15	0.15	0.15	0.30	0.30
Valine	0.28	0.14	0.28	0.14	0.14

Table 16. Effect of Phosfon and CCC on individual free amino acids expressed as per cent of the total free amino acids in leaves of Chrysanthemum morifolium 'Bluechip' three days after treatment

	10^{-5} moles of chemical per 5-inch pot				
	0	P-1(7.5)	P-2(15)	C-1(190)	C-2(380)
Alanine	13.1	6.2	7.3	6.8	8.7
Arginine	1.6	1.8	1.5	1.2	1.5
Aspartic acid	17.4	24.1	15.3	24.8	19.5
Asparagine	8.9	9.6	9.0	5.9	9.7
Glutamic acid	25.6	26.5	28.5	25.8	25.3
Glutamine	8.0	4.1	9.9	5.3	8.5
Gamma amino butyric acid	2.9	2.3	1.7	2.7	1.7
Isoleucine	3.6	3.3	4.7	2.7	3.7
Proline	5.8	5.4	7.0	9.1	10.9
Serine	8.4	13.4	10.8	12.0	6.2
Threonine	1.6	1.7	1.5	2.4	2.9
Valine	3.0	1.6	2.8	1.1	1.4

Table 17. Effect of Phosfon and CCC on free amino acids, micromoles per gram fresh weight, in leaves of Chrysanthemum morifolium 'Bluechip' six days after treatment

	10^{-5} moles of chemical per 5-inch pot				
	0	P-1(7.5)	P-2(15)	C-1(190)	C-2(380)
Alanine	0.42	0.53	0.63	0.63	0.89
Arginine	0.15	0.15	0.15	0.15	0.15
Aspartic acid	0.63	1.49	1.20	1.89	1.95
Asparagine	0.73	0.82	0.82	1.10	1.28
Glutamic acid	1.75	2.27	1.65	2.65	3.03
Glutamine	0.35	0.30	0.26	0.61	1.66
Gamma amino butyric acid	0.27	0.20	0.30	0.22	0.27
Isoleucine	0.33	0.33	0.38	0.28	0.38
Proline	0.76	0.66	0.50	0.79	1.12
Serine	1.09	1.73	1.22	1.60	1.73
Threonine	0.15	0.15	0.15	0.15	0.30
Valine	0.28	0.28	0.28	0.28	0.28

Table 18. Effect of Phosfon and CCC on individual free amino acids expressed as per cent of the total free amino acids in leaves of Chrysanthemum morifolium 'Bluechip' six days after treatment

	10^{-5} moles of chemical per 5-inch pot				
	0	P-1(7.5)	P-2(15)	C-1(190)	C-2(380)
Alanine	6.1	5.9	8.4	6.1	6.8
Arginine	2.2	1.7	2.0	1.4	1.2
Aspartic acid	9.1	16.7	15.9	18.3	15.0
Asparagine	10.6	9.2	10.9	10.6	9.8
Glutamic acid	25.3	25.5	21.9	25.6	23.2
Glutamine	5.1	3.4	3.4	5.9	12.7
Gamma amino butyric acid	3.9	2.2	4.0	2.1	2.1
Isoleucine	4.8	3.7	5.0	2.7	2.9
Proline	11.0	7.4	6.6	7.6	8.6
Serine	15.8	19.4	16.2	15.5	13.3
Threonine	2.2	1.7	2.0	1.4	2.3
Valine	4.0	3.1	3.7	2.7	2.1

DISCUSSION

Although CCC and Phosfon have been observed previously to reduce growth of chrysanthemums within several days after treatment, under the conditions of the present experiment there were no differences in growth measurements during the first week. This could possibly be due to difference in the age of the plants or temperature. Earlier experiments by the author at the University of Florida indicated that chrysanthemums are less responsive to CCC and Phosfon applications during the summer months when greenhouse temperatures of 110° to 120°F. are common. Plants used in the experiment were approximately one month older than plants used in preliminary experiments.

There was about 6 cm. of increase in height during the first six days after treatment, or approximately 25 per cent, and 5 grams or about 40 per cent increase in fresh weight of leaves. This growth rate appears to be great enough to statistically determine differences due to treatment if they occurred.

CCC had no effect on plant height at the termination of the experiment, possibly because of high temperatures. Phosfon had more dwarfing effect on growth than CCC. The low level of Phosfon reduced the height 6 cm. less than the high level of CCC. Phosfon was reported to be 25 times more effective on a molar basis than CCC (2). The molarity ratio in this experiment was 27 CCC to 1 Phosfon.

Internode length was increased by Phosfon, particularly at the low level, while CCC had no effect. Phosfon has been reported to stimulate

zinnia growth (7) and increase elongation of coleoptile segments (21). Even though the fourth internode was longer, plants treated with the low Phosfon level were shorter than non-treated plants. Thus growth retardants apparently affected new growth or that part of the stem not fully mature. Possibly, the older tissue grew at the expense of younger tissue, resulting in reduced height of the plant. Plants treated with the high Phosfon level doubled in height after treatment while untreated plants tripled in height.

Phosfon decreased the number of flower buds. The two concentrations of Phosfon affected plant height differently, but did not affect number of flower buds or flowers fully opened. Neither concentration of CCC reduced the number of flower buds nor affected plant height. Phosfon probably influenced plant height through one process and flowering through another. Since the height of the plants, but not flower number, continued to decrease with increasing rates of Phosfon, an increase of Phosfon would have little effect on the number of flowers, whereas an increase in Phosfon would decrease vegetative growth.

Phosfon reduced the number of flowers fully opened by 75 per cent, while CCC had no effect. Neither chemical greatly reduced the number of flower buds that developed, therefore Phosfon delayed opening of flowers, while CCC had no effect.

Flower diameter was affected by Phosfon in a similar manner to plant height. Plants at the high level of Phosfon had the smallest flower diameter, further indicating that Phosfon affects two different processes when it reduces growth and flowering. Once Phosfon slowed the flower initiation process, a phenomena which seems to be independent of concentration, the growth mechanism process took over and again concentration had an effect.

Inorganic analysis

Nitrogen, phosphorus, potassium, calcium and magnesium content of the stems did not vary with treatment, indicating that the growth retardants used did not cause the changes in growth and flowering by affecting the nutritional balance of the stem.

Plants receiving the high level of CCC showed a visible chlorosis at the end of the first week, but the chlorosis was apparently not due to an exit of nitrogen from the leaf. There is no evidence to show that dark green leaves attributed to growth retardants result from nitrogen accumulation in the leaves. At the termination of the experiment leaf color was the same for all treatments. The old leaves that developed the chlorosis, as well as the leaves produced after treatment did not show an effect from treatment. Chlorotic leaves had higher levels of magnesium and calcium, than the other leaves indicating that deficiency of these elements did not cause the chlorosis.

Control and CCC treated plants responded the same in potassium accumulation, both showed an increase after 24 hours, while Phosfon treated plants showed an increase at 6 hours. Potassium has been reported necessary for protein synthesis (62) and increase in cell size (63).

Phosphorus increased at the high level of CCC as did calcium and magnesium. The low level of Phosfon caused a decrease in these elements whereas they did not differ from controls at the high level of Phosfon and the low CCC level. Since growth retardants probably affect energy relationships in some manner, a more pronounced effect on phosphorus levels might be expected. However, the inorganic/organic ratio could

change without a change of total phosphorus. Wilson and Huffaker (67) reported that in severely wilted plants the concentration of most phosphorylated compounds decreased to less than half that in plants with a relative turgidity near 100 per cent, while the concentration of inorganic phosphate was not affected by moisture stress.

Iron deficiency apparently was not the cause of chlorosis as there was an increase of iron in leaves treated with CCC and the high level of Phosfon.

Although CCC did not affect growth or flowering, CCC did induce chlorosis within a week of treatment. Of the inorganic elements analyzed, calcium and magnesium content of the leaves were most influenced by CCC, but they were increased not decreased as might be expected. Phosfon, which greatly influenced plant height and flower number, had little effect on levels of inorganic elements except potassium, which decreased in the leaves the first day after treatment.

Amino acid analysis

Phosfon caused an increase in alanine six hours after treatment. Levels of alanine in CCC and control plants did not differ just as in the case of potassium. Alanine is one of the first amino acids to incorporate tagged N-15 when formed from transminations between glutamate and pyruvate (45). If nitrogen metabolism were altered, alanine probably would be one of the first to respond.

Asparagine and glutamine increased in many plants because of nutritional deficiencies (11,16,18). This could result from protein breakdown or lack of protein synthesis. There does not appear to be any clear distinction between the effect of the two growth retardants on the distribution of these amides. Plants treated with Phosfon and CCC did not increase the amides that often follow poor growing conditions.

Phosfon and CCC treated plants contained more aspartic acid 24 hours after treatment than controls. Aspartic acid was reported to be one of the chief means by which ammonia is incorporated into the metabolic pathways of plant cells (64). There was no difference in content of glutamic acid between control and treated plants. When N-15 ammonia is taken up by cells, it is usually incorporated most readily into glutamate, probably due to a vigorous amination of alpha ketoglutarate.

Proline increased then decreased when plants were treated with the high Phosfon level. CCC treated plants reacted similarly but to a smaller degree. Steward and Bidwell (54) and Steward and Pollard (55) reported that the specific activity of proline and hydroxyproline in protein was closely related to that of C-14 in proline free in the cell, but that of glutamic acid, aspartic acid and threonine was not. Carbon could be directly incorporated into the protein from the free proline of the cell. Steward further stated that the protein moiety which incorporated C-14 directly from proline does not participate in metabolic turnover. Olsen (46) fractionated cultured tobacco cells into a protoplasmic fraction, and cell-wall extracted fraction and a cell-wall residual fraction, and found that proline is rapidly incorporated into protoplasmic protein and much more slowly into the cell-wall fraction. Olsen also reported a rapid turnover of protoplasmic protein, and little, if any, turnover of cell-wall protein. An accumulation of free proline caused by Phosfon treatment is an indication that the incorporation of free proline into protoplasmic protein is blocked. Fowden (15) reported that histidine, tyrosine, cysteine and methionine are rarely detected in the free state in plant extracts unless the plant has been subjected to conditions favorable for rapid protein breakdown. None of these acids were found in chrysanthemum extracts indicating that protein breakdown did not occur.

Although treatment of plants with CCC caused a change in inorganic element and amino acid content, there was no effect of CCC on flowering or growth of plants. The compound evidently disturbed the normal functions of the plant but the plant was able to continue its functions without reducing growth or flowering.

The two growth retardants did not have the same effect on the distribution of inorganic elements or amino acids in plants, nor on vegetative or flowering responses.

The plants stabilized the increased quantities of proline by the sixth day indicating that even though the growth is reduced later than the sixth day after treatment with Phosfon the disturbances inside the cells are temporary.

SUMMARY

A study was made of the effects of Phosfon and CCC on the growth, flowering and chemical composition of Chrysanthemum morifolium 'Bluechip.'

Growth measurements used were the height of stems, fresh and dry weight of leaves and stems and length and diameter of the fourth internode. Flower measurements included the number of flower buds, number of flowers fully opened and the flower diameter. Determinations were made of the free amino acids, total nitrogen, potassium, phosphorus, calcium and magnesium.

CCC had no effect on either growth or flower measurements. However, treatment with CCC did increase calcium, magnesium, iron, asparagine and glutamine, glutamic and aspartic acid. The greatest change in amino acid content caused by treatment with CCC was an increase of serine. CCC evidently disturbed the normal functions of the plant but the plant was able to continue its functions without reducing growth or flowering.

Phosfon reduced plant height, number of flower buds, number of flowers fully opened and flower diameter. Treatment with Phosfon reduced the content of potassium in leaves and increased alanine and aspartic acid. Proline was greatly increased in leaves of plants treated with Phosfon. The accumulation of proline is an indication that Phosfon may restrict the entrance of free proline into protoplasmic protein.

The high level of Phosfon reduced plant height and flower diameter more than the low level of Phosfon, but the increased concentrations did

not reduce the number of flower buds or flowers fully opened, suggesting that the growth retardant affected flowering through one process and growth through another.

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BIOGRAPHICAL SKETCH

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This dissertation was prepared under the direction of the chairman of the candidate's supervisory committee and has been approved by all members of that committee. It was submitted to the Dean of the College of Agriculture and to the Graduate Council, and was approved as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

December 19, 1964

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